

**STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE
INVESTIGATIONAL NEW ANIMAL DRUG (INAD)
EXEMPTION FOR AQUI-S[®]E (eugenol)
(INAD #11-741)**

Sponsor:

U.S. Fish and Wildlife Service, Division of Fish Hatcheries

Sponsor Signature

Date Approved

Manufacturer:

Western Chemical, Inc. /AQUI-S New Zealand, Ltd.
1269 Lattimore Road
Ferndale, WA 98248

Facility for Coordination of AQUI-S[®]E INAD:

Aquatic Animal Drug Approval Partnership Program
U.S. Fish and Wildlife Service
4050 Bridger Canyon Road
Bozeman, Mt 59715

Proposed Starting Date: August 1, 2009

Proposed Ending Date: July 31, 2012

Study Director: Mr. Jim Bowker (FWS/AADAP)

Study Director Signature

Date

Clinical Field Trial Location:

Type or Print Facility Name

Investigator_____

Type or Print Name

Investigator Signature

Date

STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR AQUI-S®E (eugenol) UNDER INAD #11-741

I. STUDY IDENTIFICATION AND TITLE

Clinical field trials to determine the efficacy of AQUI-S®E as an anesthetic for use in a variety of fish species. INAD #11-741

II. SPONSOR

Dr. David Erdahl, U.S. Fish and Wildlife Service, Branch Chief, Aquatic Animal Drug Approval Partnership (AADAP) Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9904; Fax: 406-582-0242; Email: dave_erdahl@fws.gov

Manufacturer: Western Chemical, Inc./AQUI-S New Zealand, Ltd.
1269 Lattimore Road
Ferndale, WA 98248

Contact person at Western Chemical Inc.:

Jason Montgomery
Phone: 1-800-283-5292 extension 4175; 360-312-4175
Email: jasonm@wchemical.com

Study Director: Mr. Jim Bowker, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership (AADAP) Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9910; Fax: 406-582-0242; Email: jim_bowker@fws.gov

Principal Clinical Field Trial Coordinator: Bonnie Johnson, USFWS - AADAP

INAD Study Monitors: See Appendix II for names and addresses.

III. INVESTIGATORS/FACILITIES

See Appendix IIIa for names and addresses

IV. PROPOSED STARTING AND COMPLETION DATES:

Proposed Starting Date: August 1, 2009

Proposed Completion Date: July 31, 2012

V. BACKGROUND/PURPOSE

A. Background Information:

The use of anesthetics is an important tool with broad application to fisheries management programs. Most often, anesthetics are used to reduce stress associated with the handling and/or transportation of fish. Anesthetics are widely used both in the culture of captive populations, and in field situations that involve the management of wildstock fish populations. Although a number of compounds have been used in the past, currently, the only approved anesthetics for use on fish are Finquel and Tricaine-S, (active ingredient tricaine methanesulfonate, NADA 042-427 and ANADA 200-226, respectively). Finquel and Tricaine-S both have a withdrawal period of 21 days. This restriction requires that potential food fish must be held for a minimum of 21 days following treatment before they can be released for legal harvest or slaughtered. While both of these products have been found to be effective anesthetics for use in fish, their required 21-day withdrawal period severely restricts approved use in many situations. In contrast, a zero-withdrawal (or immediate release) anesthetic would allow food fish to be released, stocked, or slaughtered “immediately” following treatment. In numerous fisheries management programs, and particularly those involving wildstock population assessment and evaluation, there is a critical need for such an anesthetic. AQUI-S[®]E has been developed in New Zealand as an anesthetic for use on food-fish with no withdrawal period. The active ingredient in AQUI-S[®]E, eugenol, is used in perfumeries, flavorings, essential oils, and in medicine as a local antiseptic and anesthetic.

B. Purpose of INAD:

The purpose of this compassionate INAD for AQUI-S[®]E is to develop clinical efficacy field trial data that will be used to determine the most appropriate treatment regime for AQUI-S[®]E for use as an anesthetic in a variety of fish species. These data will be used to support a new animal drug application (NADA) for AQUI-S[®]E.

The U. S. Fish and Wildlife Service (USFWS) anticipates requesting the U. S. Food and Drug Administration (FDA) to grant extensions of this INAD for additional years. The USFWS believes that data from at least 1-3 treatment seasons will be required in order to adequately assess the efficacy of AQUI-S-E as an anesthetic for use in fish, and to collect sufficient data to support a NADA.

VI. SPECIFIC OBJECTIVES

The two major objectives of this study protocol are as follows:

1. Collect scientific data necessary to establish the effectiveness of AQUI-S[®]E as an anesthetic in a variety of fish species under a variety of environmental conditions (e.g. temperature, water hardness, pH, turbidity, etc)
2. Provide an opportunity for fish culturists and fisheries managers to legally use AQUI-S[®]E as an anesthetic so that they can maintain and manage healthy stocks of fish during the period of time necessary for collection of efficacy, safety, and residue data required for an NADA for AQUI-S[®]E in fish

VII. MATERIALS

A. Test and control articles:

1. Drug Identity

a. Active ingredient

Chemical Name: eugenol [2-methoxy-4-(propenyl) phenol]

C.A.S. Registry No.: 97-53-0

Molecular Formula: $C_{10}H_{12}O_2$

Appearance: Yellow, viscous liquid

Odor: Spicy, pungent, clove-like

Specific Gravity: 1.075 to 1.095

b. Strength and dosage form

AQUI-S[®]E is 50% eugenol (active ingredient). Fish are exposed by immersion bath.

c. Manufacturer, source of supply

Western Chemical, Inc./AQUI-S New Zealand, Ltd.
1269 Lattimore Road
Ferndale, WA 98248

Contact person at Western Chemical, Inc. is:

Jason Montgomery
Phone: 1-800-283-5292 extension 4175; 360-312-4175
Fax: 360-384-0270
Email: jasonm@wchemical.com

The shipment procedure for AQUI-S[®]E is as follows: AQUI-S New Zealand, Ltd. to Investigators (See Section VII.A.6 Accountability [page 5] for details and Appendix IIIa for names and addresses of Investigators).

2. Verification of drug integrity/strength:

The Manufacturer (AQUI-S New Zealand, Limited) purchases ingredients for AQUI-S[®]E using specification of the Food Chemicals Codex for eugenol and U.S. Pharmacopoeia for the inert ingredients. The vendors assay these ingredients and certify they meet purchase specifications. The manufacturer also contracts Industrial Research, Ltd., (an ISO 9001 certified analytical lab) to analyze the eugenol by proton nuclear magnetic resonance (NMR) and confirm it meets purchase specifications.

To achieve 50% active ingredient concentration, the manufacturer weighs the eugenol

then blends it with an equal weight of inert ingredients. The manufacturer records the weight of ingredients for each batch and total weight of the mixture. The date of manufacture, as well as batch and lot numbers are also recorded. Samples of batches are analyzed by Industrial Research, Ltd., using NMR to verify the integrity and strength of the final product.

AQUI-S New Zealand, Ltd. will provide the analytical data necessary to establish the purity of each lot/batch of AQUI-S[®]E supplied. The lot number and date of manufacture for each batch of AQUI-S[®]E will be placed on the label of each container. The form "Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals" (Form AQSE-1) will clearly identify the lot number and date of manufacture of AQUI-S[®]E shipments. If the integrity of the AQUI-S[®]E is compromised (i.e., by spilling or contamination of the stock container) the event will be carefully recorded, dated, and signed in the Drug Inventory Form (Form AQSE-2). The Study Monitor assigned to the Investigator involved will be immediately notified and the remaining material will be returned to the Study Monitor along with the properly recorded Form AQSE-1.

3. Storage Conditions

AQUI-S[®]E will be stored in the original container supplied by the Manufacturer with the appropriate investigational label attached. AQUI-S[®]E has high stability and should be stored at room temperature in a dry location away from direct sunlight. AQUI-S[®]E should be stored in a secure location such as in a locked cabinet.

4. Handling Procedures

Each Study Monitor and Investigator will be required to have a current copy of the Material Safety Data Sheet (MSDS) for AQUI-S[®]E (Appendix IV). Each person involved with the study and each person who may be present during the use of AQUI-S[®]E shall be required to read the MSDS. Safety precautions as outlined in the MSDS will be followed at all times when working with AQUI-S[®]E. Standard laboratory equipment such as gloves, lab coats or aprons, eye protection, etc., will be worn at all times.

5. Investigational labeling

A copy of the label to be attached to each container of AQUI-S[®]E is provided in Appendix V. It is the responsibility of the Investigator to ensure proper labeling of all containers of AQUI-S[®]E.

6. Accountability

AQUI-S New Zealand, Ltd. will be the sole supplier of AQUI-S[®]E to all Investigators under this INAD.

1. USFWS and Non-USFWS Facilities

Immediately upon receiving an order/shipment of AQUI-S[®]E, the Investigator will complete Form AQSE-1 "Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals". The investigator will archive the

original in the facilities INAD file, and send a copy to his/her Study Monitor. Both the Investigator and the Study Monitor are required to sign Form AQSE-1. The Study Monitor will then forward a copy to the AADAP Office. The AADAP Office will archive one copy, and send one copy of Form AQSE-1 to FDA. Arrangements should be made between Investigators and Study Monitors to insure completed Form AQSE-1s are received by the AADAP Office within 10 days of drug receipt.

All Investigators are also responsible for maintaining an accurate inventory of AQUIS[®]E on-hand. A Drug Inventory Form (Form AQSE-2) will be supplied to each Investigator. Each time AQUIS[®]E is used, it must be reported by the Investigator on Form AQSE-2.

At the conclusion of the study, all remaining AQUIS[®]E will be shipped to the Study Monitor along with the properly recorded Drug Inventory Form (Form AQSE-2). The Study Monitor will then verify the Drug Inventory Form against the quantity of AQUIS[®]E remaining. All remaining AQUIS[®]E will then be returned to the Manufacturer.

7. Preparation Procedures

AQUIS[®]E will be prepared according to label directions for normal use. This includes accurately weighing out the calculated amount of AQUIS[®]E to obtain the desired dose, adding freshwater to establish at least a 10-fold dilution, vigorously shaking the solution to ensure thorough mixing, and then uniformly mixing the solution in the treatment tank water. Note: Do not add AQUIS[®]E directly to treatment tank water.

B. Items Needed for Treatment, Sample Collection, Observations, Etc.:

Treatment and diagnostic equipment should include a balance, graduated cylinder or flask, treatment tank, recovery tank, thermometer, stop watch, and a dissolved oxygen meter.

VIII. EXPERIMENTAL UNIT

The experimental unit in this clinical field trial will consist of a contained or isolated group of fish. This will generally be a group of fish contained in a tank, raceway, or pond. In some cases, the experimental unit may be individual animals.

IX. ENTRANCE CRITERIA

A. Facilities/Investigators

The proposed facility and the Investigator must be listed in Appendix IIIa of this Study Protocol before AQUIS[®]E can be ordered and dispensed under this INAD. Last minute deviations can be requested by the Sponsor, Study Director, or Investigator in case emergency use-pattern needs should arise (See Section XX).

B. The characteristics of the study animals (species, number, etc.) is presented in Appendix VIb.

C. Environmental conditions

Environmental conditions will be variable and include a broad spectrum of temperatures and water quality parameters. Environmental conditions will be reported on Form 3.

D. Ability of investigator to fulfill all the requirements of the Study Protocol

See Appendix IIIb for example of knowledge required of hatchery managers (i.e., Investigators).

Prior to initiating each treatment event, the Investigator must first complete Form AQSE-W, "Worksheet for Designing Study Protocol" that pertains to each specific treatment event. The worksheet should be filled out, signed, and sent by Fax or email to the Study Monitor. The Study Monitor will review the planned treatment (worksheet), sign it, and forward (Fax) the paperwork to the AADAP Office. The AADAP Office will then review the worksheet, assign the approved treatment a Study Number, and then notify both the Investigator and the Study Monitor of the assigned number and approval to proceed. In most cases, this entire process should be able to be accomplished within a single working day. The Investigator should record the assigned study number on Form AQSE-3, as well as on any additional correspondence regarding that specific treatment event. If for some reason the Investigator is unable to reach his/her Study Monitor with regards to worksheet approval, and the need for treatment is immediate, the Investigator should contact the AADAP Office for a study number and permission to proceed.

X. TREATMENT GROUPS

A. A treatment group or experimental unit may be an entire tank, pond, raceway, or group of fish, or it may be individual animals.

B. Non-treated control groups will not be a requirement for clinical field trials evaluating the efficacy of AQUI-S®E as an anesthetic. As the primary use of AQUI-S®E will be to facilitate handling and reduce stress to fish when they are being handled, untreated controls would in most cases be extremely impractical, as well as detrimental to fish

health. However, Investigators are encouraged to record observations with respect to the behavior and physiological state of fish prior to AQUI-S®E treatment. This information will provide a "pseudo-control" as to fish condition without, or prior to, AquI-S®E treatment.

Although untreated control groups are not a required element of treatment under this INAD exemption and are at the discretion of the Investigator, they are strongly encouraged whenever circumstances permit. Control groups are extremely important to not only document response to treatment, but also to validate potential adverse reactions in treated animals. Assignment to control and treatment groups should be random and designed to avoid bias. It is important that all fish are treated in a similar fashion. If fish are physically moved into separate test groups or different rearing units, caution should be used so that handling and rearing conditions are as similar as possible. Control fish should be kept under conditions as similar as possible to treated fish for valid comparison. Use of control groups will ensure that results of efficacy studies provide useful information that will support a NADA.

Blinded studies can reduce bias in data collection. Whenever possible, investigators should consider methods by which treatment response observations are recorded by individuals who are unaware which fish have been treated, at what dosage levels fish have been treated, and/or which fish are controls.

XI. TREATMENT SCHEDULES

A. Route of administration

AQUI-S®E will be administered as a static immersion bath treatment. AQUI-S®E will be prepared according to label directions for normal use. Dependant upon the desired dosage, the calculated amount of AQUI-S®E necessary to achieve the target dosage of eugenol should be accurately weighed, and diluted a minimum of 10-fold with fresh water. Following vigorous shaking to ensure thorough mixing, the solution should be uniformly distributed and mixed with treatment container water. Note: Do not add AQUI-S®E directly to treatment tank water.

B. Dose to be administered

AQUI-S®E should be applied as a static immersion bath at eugenol concentrations ranging from 10 - 100 mg/L (**note: AQUI-S®E is 50% eugenol as the active ingredient**). Within this range, the actual concentration applied will be at the discretion of the Investigator. Dosage will likely vary with respect to species, water temperature, and level of anesthesia desired.

C. Dosing interval and repetition

AQUI-S®E will be applied as a single treatment event, and will not require repeated treatments.

D. Duration of treatment

Treatment duration will be variable, and dependant on species, water temperature, and level of anesthesia required to meet handling requirements. For example, treatment to facilitate fin-clipping would likely require light sedation (i.e., sedation to a handleable condition) and a relatively short treatment duration (1-5 minutes), whereas treatment to facilitate implantation of radio transmitters would likely require deep sedation (i.e., sedation to an anesthetized condition) and a relatively long treatment duration (5-10 minutes). In most cases, it is expected that duration of treatment will be from 1-15 minutes. After completion of treatment and handling, fish should immediately be placed fresh water.

E. Disposition of anesthetic solution

If at all possible, discharge of anesthetic solution remaining in the treatment containers following completion of treatment should be to the ground. If ground discharge is not possible, anesthetic solution may be released/mixed with facility effluent. In situations where minimal dilution of anesthetic solution occurs prior to release to public surface

waters, a pulsed-release of anesthetic solution should be employed to minimize discharge levels.

F. Detailed procedures for drug administration

Standard laboratory equipment such as gloves, lab coats or aprons, eye protection, etc. should be worn at all times when working with AQUI-S®E. The amount of AQUI-S®E necessary for each treatment should be accurately weighed immediately prior to treatment. To aid in the uniform distribution of chemical, AQUI-S®E should be diluted at least ten-fold in a fresh water stock solution prior to treatment.

G. Permissible concomitant therapy

Since efficacy data are being collected during the INAD process, there should be little or no concomitant therapy. Preferably, there should be no other therapy during a period extending from 2 weeks prior to treatment to 2 weeks after treatment. Investigators must be prepared to minimize changes in fish cultural procedures or environmental conditions, and apply no other treatments following treatment with AQUI-S®E. However, if concomitant therapy is required in order to protect valuable fish stocks, it should be fully documented and the efficacy data from the AQUI-S®E treatment involved should be appropriately labeled.

XII. TREATMENT RESPONSE PARAMETERS

The collection and reporting of source data begins with the decision to treat valuable fish based on hatchery records or field management practices that indicate treatment is warranted. Daily morbidity and mortality records, case history records, as well as any extenuating or mitigating circumstances that may affect treatment response need to be documented. All pertinent treatment response parameters should be reported on Form AQSE-3. Treatment response parameters that should be addressed include the following:

1. Primary Parameters

The primary treatment response parameters in this study will be a reflection of the physiological condition of fish following treatment with AQUI-S®E. The primary physiological conditions evaluated will include when a fish is considered to be: 1) "handleable"; 2) "anesthetized"; 3) euthanized; or 4) "recovered". In most cases, dependent upon level of anesthesia desired, a study will involve either handleable or anesthetized, and recovered.

Handleable

A fish will be considered handleable when it begins to lose equilibrium, and when it has lost reactivity to most external stimuli with the exception of strong pressure. This condition generally occurs after a fish stops avoiding obstacles in its path, and before it completely loses equilibrium. As a general rule, a fish will be considered handleable when it can be held underwater for several seconds without great difficulty. This is similar to Stage 2 anesthesia as described by Summerfelt and Smith, 1990.

Anesthetized

A fish will be considered anesthetized when it loses all reflex activity. This condition generally occurs after a fish has completely lost equilibrium. As a general rule, a fish will be considered anesthetized when it can be easily held out of water, and when lifting the operculum and touching the gill lamellae does not elicit a reflexive “cough” within 5 seconds. This is similar to Stages 4-5 anesthesia as described by Summerfelt and Smith, 1990.

Euthanized

A fish will be considered euthanized when all opercular movements have ceased for a period of at least 2 minutes. Note: Euthanized fish must not be sent to slaughter or be otherwise available for food.

Recovered

A fish will be considered recovered from anesthesia when it exhibits normal swimming behavior, including avoidance of obstacles. For this study, the fish must recover in less than 30 minutes of exposure to fresh water to be considered “recovered”.

As a result of the potential diversity of treatment conditions and unique treatment response parameters (e.g. specific level of anesthesia desired) that may be involved in these studies, Investigators are encouraged to provide detailed descriptions of all study variables, including specific definitions/descriptions of level of anesthesia and criteria used to establish anesthesia levels. Investigators may also choose to create their own forms for purposes of recording source data under this INAD. **Supplementary data forms should be attached to Form AQSE-3.**

2. Secondary Parameters

Secondary parameters include general observations on fish behavior and response to routine culture/management activities. Secondary parameters would include such responses as feeding activity, feed consumption, apparent level of stress, negative fish behavior, post-release behavior, etc.

3. Adverse Reactions

Any adverse reaction to treatment should be reported immediately to the Study Monitor, who will in turn notify the Study Director. Such responses might include changes in water quality, extremely negative responses/behavior by the fish, or hazards to the applicator. Although AQUI-S®E is currently approved in New Zealand and Australia and has been used fairly extensively with beneficial effect, it is possible adverse reactions may occur under certain environmental conditions or with respect to specific species/strains of fish. Careful observation of all treated fish for signs of any adverse reaction to treatment is extremely important, and all observations of adverse reactions should be documented. If any signs of drug toxicity are detected, they should also be documented and immediately reported to the Study Monitor, who will in turn notify the Study Director.

Note: Investigators are strongly encouraged to record observations/comments with

respect to all phases of treatment. This may include a description of events before, during, and post-treatment. All extenuating or mitigating treatment circumstances need to be described in detail. Such information is imperative so that accurate study/data analysis can be performed.

XIII. FORMS FOR DATA COLLECTION

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the AQUI-S®E INAD will need to complete the following forms:

- Form AQSE-W. Worksheet for Designing Individual Field Trials
- Form AQSE-1. Report on Receipt of Drug
- Form AQSE-2. Drug Inventory Form for use of AQUI-S®E under INAD #11-741
- Form AQSE-3. Results Report Form for use of AQUI-S®E under INAD #11-741

Copies of these forms are attached to this Study Protocol.

XIV. RECORD KEEPING PROCEDURES

The data should be recorded in permanent ink (preferably black). The data should be recorded on the official data record forms at the time the observations are made. The raw data should be original, i.e., they should be the first recording of the observations, rather than a transcription of original observations to another data sheet. Each original data sheet should be legibly signed and dated by the person making the observation and recording the entry. If more than one person makes and records the observations, entries should be properly attributed to each person. The data should be accurate and legible. If a mistake is made, it should be crossed out using a single strike-through and the correct data should be recorded next to it; each change to the raw data should be initialed and dated by the person making the change, and a statement should be provided explaining why the change was made. If the data sheet needs to be copied, all data should be transferred, including the properly noted changes; the original record should be retained and submitted with the revised copy, along with a memo explaining the reason for the copying.

XV. DISPOSITION OF INVESTIGATIONAL ANIMALS

Animals that die during treatment should be disposed of by burial or incineration. All treated fish must be held for at least 72 hours following treatment with AQUI-S®E before they are stocked or allowed to enter the food chain.

No withdrawal period will be required for fish that will not be catchable for 72 or more hours after release or are illegal for harvest during that 72 hour period. No withdrawal period shall be required for dead fish that will be buried or rendered into non-edible products. Note: Euthanized fish must not be sent to slaughter or be otherwise available for food.

The Investigator must record the disposition of all treated fish on Form AQSE-3.

XVI. DISPOSITION OF INVESTIGATIONAL DRUG

AQUI-S®E will be used only in the manner and by the individuals specified in the Study Protocol.

At the conclusion of the study, all remaining AQUI-S®E will be shipped to the Study Monitor along with the properly recorded Drug Inventory Form (Form AQSE-2). The Study Monitor will then verify the Drug Inventory Form against the quantity of AQUI-S®E remaining. All remaining AQUI-S®E will then be returned to the Manufacturer. The investigational drug may not be redistributed to others not specified by the protocol and may not be retained by the Investigator after completion of the study.

XVII. DATA HANDLING, QUALITY CONTROL, MONITORING, ADMINISTRATIVE RESPONSIBILITIES

A. Drug distribution

See Section VII.A.6. Accountability (page 5) for information and details.

B. Study Monitors

The Study Monitors are generally fish health professionals with experience in diagnosing and treating fish diseases. There is one Study Monitor assigned to each facility within the USFWS that is covered by the AQUI-S®E INAD. Non-Service facilities must have a similar Study Monitor - Investigator relationship in place. A list of Study Monitors, along with addresses and phone numbers, can be found in Appendix II. The Study Monitors are responsible for supervision of the trials, adherence of the Investigator to the Study Protocol, and inspection of the site.

C. Special equipment and materials

Most of the equipment and materials required for this study (with the exception of the AQUI-S®E itself) are already available at each participating facility. The use of anesthetics to aid in the handling of fish is a common occurrence at most fish hatcheries and in many fisheries management programs. Fish hatchery managers and fisheries managers (i.e., Investigators) are well trained and well equipped to supervise these procedures (see Appendix IIIb). If any additional equipment or materials are required, they will be provided by the Study Monitors (See Section VII.B. Items needed for sample collection, observations, etc., page 5).

D. Administrator of the drug

AQUI-S®E will be administered directly by the assigned Investigator (fish hatchery manager or fisheries manager) or under the Investigator's direct supervision (see Appendix IIIa for names). AQUI-S®E will be maintained in a secure location, and only the Investigator or a person under his/her direct supervision will have access.

E. Drug accountability records

See Section VII.A.6. Accountability (page 5) for details and Form AQSE-W, Form AQSE-1, Form AQSE-2, and Form AQSE-3 for actual forms to be used in the study.

F. Recording observations

The Investigator or a person under his/her direct supervision will be responsible for implementing the Study Protocol, making observations, collecting samples, and recording data during the clinical field trials. After the data have been collected and recorded on the forms, the Investigator will send the data to the Study Monitor who will ensure that all required information is provided. The Study Monitor will in turn send the data to the Study Director. The Study Director will analyze and summarize the data and prepare an annual report that will be submitted to the FDA. **Note: If the Study Monitor does not think all required information has been provided, or forms have not been satisfactorily completed, he/she should contact the Investigator and rectify the situation before forwarding the package to the Study Director.**

G. Data storage

The Investigator is responsible for complete and accurate data collection. The investigator is also responsible for archiving a complete set of all original data for at least 2 years following the completion of a study. The Investigator is also responsible for archiving a complete set of all original data. A copy of Form AQSE-1 should be sent immediately to the Study Monitor, who will in turn forward a copy to the Study Director. Original raw data on Form AQSE-2 should be retained by the Investigator until completion of the calendar year, at which time copies should be sent to the Study Monitor. Original raw data on Form AQSE-3 should be retained by the Investigator until completion of the study, at which time copies should be sent to the Study Monitor. Study Monitors should carefully check each set of data for accuracy and completeness. If there are any discrepancies in the data, the Study Monitor should contact the Investigator immediately to rectify the problem. After review, Study Monitors should forward all data to the Study Director. As stated above, a complete set of raw data should be archived by the Investigator. All data should be stored in a secure place. Another complete data set (copies) will be archived by the Study Director.

Form AQSE-3 Results Report Form is to be completed no later than 30 days after a course of therapy is completed. The purpose of this form and supplementary data is to document the results of the treatment. In addition to the data solicited by the form, attach original source data that may have been collected to document any treatment effect.

XVIII. PLANS FOR DATA ANALYSIS

Data analysis will be completed by the Field Trial Coordinator located at the AADAP Office. Data from the treatment year will be summarized through tabulation and appropriate statistical analysis. An annual INAD report will be prepared and submitted to the FDA. This submission may include a request for an extension of the INAD based on the data collected during that year. When sufficient data are collected, the entire INAD data set will be summarized in a final report for submission to support a full NADA.

XIX. PROTOCOL AND PROTOCOL AMENDMENTS

A signed copy of the Study Protocol must be retained by each Investigator. At any time before the study begins, desired changes in the Study Protocol should be brought to the attention of the Study Director. The desired changes will be fully described in the form of an amendment along with the reason for the change. The amendment will be signed by the Sponsor (or its representative). Copies of the signed amendment will be attached to each copy of the Study Protocol. **Investigators will be liable for non-compliance violation if drugs are used without a Study Protocol or differently than specified in the Study Protocol, if forms are not filed on time, or if the study data are not properly collected, maintained, and reported.** The Study Monitor is responsible for ensuring that all INAD procedures are being followed as defined by the Study Protocol.

XX. PROTOCOL DEVIATIONS

Deviations from the established Study Protocol occasionally cannot be avoided. If deviations occur, the Study Monitor should be contacted immediately for advice. **Protocol deviations should be fully documented and should be accompanied by a written explanation of what happened, why, and what steps were taken to mitigate the deviation.** Deviation statements should be signed and dated. These statements should be forwarded to the Study Monitor along with the quarterly data summaries, and ultimately be submitted to the Study Director.

References

Summerfelt, R.C. and L.S. Smith. 1990. Anesthesia, surgery, and related techniques. In: Methods for Fish Biology. C.B. Schreck and P.B. Boyle editors. American Fisheries Society. Bethesda, Maryland. pp. 213-272